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OSMOREGULATION OF *CRANGON SEPTEMSPINOSA* SAY (CRUSTACEA: CARIDEA)[1]

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Species of estuarine Crustacea are subjected to an environment in which fluctuations in salinity may be sudden or continuous. Other factors, such as temperature and dissolved oxygen, will normally vary with salinity. Any one or more of these factors may influence the process of osmotic regulation (Kinne, 1964). Although a causal relationship may be expected to exist among these different factors, this has not as yet been fully explained (Lange, 1968).

Numerous osmoregulatory studies have been performed on a variety of penaeid and palaemonid Crustacea. Panikkar (1941) observed that *Palaemonetes varians, Palaemon serratus* (= *Leander serratus*) and *Palaemon elegans* (= *L. squilla*) were capable of hypotonic regulation in normal sea water and of hypertonic regulation in diluted media. Similar regulatory patterns have been reported for *Metapanaeus monoceros* (Panikkar and Viswanathan, 1948), *M. dobsoni, Penaeus indicus, P. carinatus* (Panikkar, 1951), *Palaemonetes intermedius* (Dobkin and Manning, 1964), *Penaeus aztecus, P. duorarum* (Williams, 1960), and *Palaemon macrodactylus* (Born, 1968).

Considerable attention has been given to the caridean, *Crangon crangon* L., a euryhaline species which ranges from the White Sea (Wollebaek, 1908) to the Moroccan coast of Africa (Nouvel and Panouse, 1965). Caudri (1937) provided evidence that the osmoregulatory capacity of *C. crangon* is related to temperature. Mathias (1938) observed moderate survival to low salinities with death ensuing after 7–8 hours of exposure to freshwater. Investigations by Broekema (1941) indicated that optimum salinity varied with age of the specimens and that hypo- and hypertonic osmoregulation was more efficient at higher temperatures and inhibited at low temperatures. Lloyd and Yonge (1947), in field and laboratory observations, concluded that males cannot withstand as low salinities as females and that optimal salinity, at 15° C, is higher for males. Flügel (1960, 1963) showed that osmotic resistance to low and high salinities (1–5‰ and 30–90‰) is higher at 5° C than at 15° and 20° C. The efficiency of osmoregulation does, however, decrease at temperatures below 5° C. This loss of resistance is correlated with the fact that in the northern Baltic Sea, *C. crangon* is incapable of survival in low salinity water at temperatures near freezing.

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Crangon septemspinosa Say is a good example of a euryplastic inhabitant of Atlantic east coast estuaries. Within its geographic range from the Gulf of St. Lawrence (Squires, 1965) to eastern Florida (Rathbun, 1929; Whitely, 1948; Williams, 1965), it has been collected within temperature and salinity ranges of −3° to 25° C and 3.4 to 32‰, respectively (Stickney, 1959; Price, 1962; Haefner, P. A., Jr., unpublished). A recent multivariate study (Haefner, 1969) has delineated the influence of temperature, salinity and temperature-salinity interaction on survival of this species. The present study was inaugurated in order to determine if *C. septemspinosa* does regulate its internal salinity, and if so, to what extent under the influence of a variety of temperature and salinity combinations.

Methods

Shrimp used in this research were collected at Lamoine Beach, Maine, and transported in natural seawater of 29–31‰ to experimental facilities. After thermal acclimation, the shrimp were transferred to 30‰ seawater and maintained on a mixed diet of haddock, brine shrimp and blue mussel. A holding time of at least 7 days, and usually longer, was adhered to prior to subjecting the shrimp to experimental conditions.

The various salinities used in the research were made with commercially available synthetic sea salts (Segedi and Kelly, 1964) and determined by the low precision method of Strickland and Parsons (1965). The pH was monitored with a portable Beckman meter.

Temperature was maintained in a constant temperature room and monitored with a Taylor recording thermometer.

Freezing points of blood and seawater were determined with a thermoelectric cryoscope (Clifton Technical Physics, Wannamassa, New Jersey) reading in centigrade degrees. After the animal was blotted dry with filter paper, a blood sample was obtained by inserting a micropipet into the pericardial cavity accessible through the membrane between the posterior edge of the carapace and the first abdominal segment. Hemolymph was drawn into the oil-filled pipet and two 1-nanoliter volumes were transferred to the cryoscope platform for reading. Samples of seawater were handled in a similar fashion.

Survival studies on *C. septemspinosa* indicated that definitive mortality occurred by the fourth day when the shrimp were subjected to changes in salinity (Haefner, 1969). In an effort to determine the time involved for the surviving organism to equilibrate osmotically with its experimental environment, and the extent of the steady state, the following experimental routine was performed. Shrimp were transferred from the holding aquarium water (30‰) to experimental seawater of 15‰, at 5° C. Freezing point determinations were made on the blood of at least 9 individuals of each sex (juvenile, male, non-ovigerous female) selected prior to transfer and at 1, 2, 4, 8, 12, 18, 24, 48 and 96 hours after exposure to the new salinity. After this initial pattern was observed, the number of sampling times was reduced for the transfer of shrimp from 30‰ to 45‰, at 5° C.

The major part of this study was relegated to analysis of freezing point depressions of shrimp subjected to 5 salinities (15, 25, 30, 35, 45‰) and 2 temperatures (5, 15° C) for periods greater than 96 hours. Extensive mortality prevented the collection of sufficient data for 5‰ even when a stepwise salinity transfer was employed.

The freezing point depression data were treated according to Tan and Van Engel's (1966) modification of the graphic method of Hubbs and Hubbs (1953). Such a chart indicates if there are significant differences between the means of juvenile, male and female shrimp for each temperature-salinity combination as well as differences between the shrimp subjected to different salinities and for different periods of time. Significant difference in the means at the 1% level occurs if the solid, cross-hatched or stippled bars ($t_{0.05}\chi$ standard error on either side of the mean) do not overlap one standard deviation on either side of the mean (hollow bars). Significant difference at the 5% level occurs if the patterned bars overlap without reaching one of the means. There is no difference in the means if the patterned bars overlap the means.

Results

Experimental acclimation times

Sand shrimp, transferred acutely from one salinity to another, experience changes in the osmoconcentration of the hemolymph which are related to the osmotic strength of the receiving media. Although the trend is a gradual change in osmoconcentration toward that of the new salinity, there was a noticeable oscillation in the pattern prior to the attainment of a new steady state.

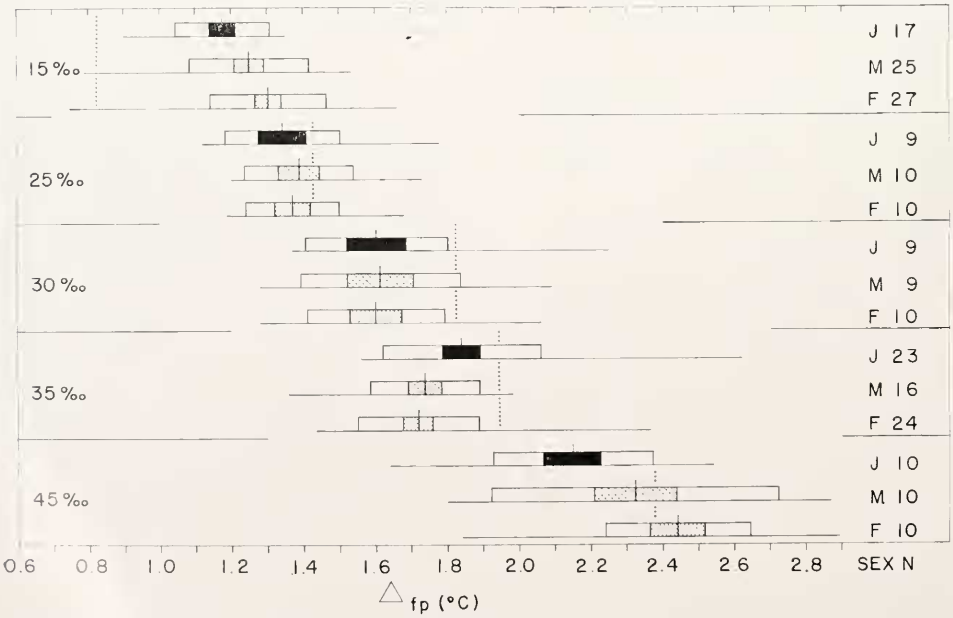

FIGURE 1. The means, standard deviations and $t_{0.05}$ standard errors for freezing point depressions of blood of juvenile (J), male (M) and female (F) sand shrimp exposed to six salinities for longer than 4 days at 5° C. Number (N) of shrimp sampled is indicated in right margin. Dotted lines indicate mean freezing point depressions of 5 salinities.

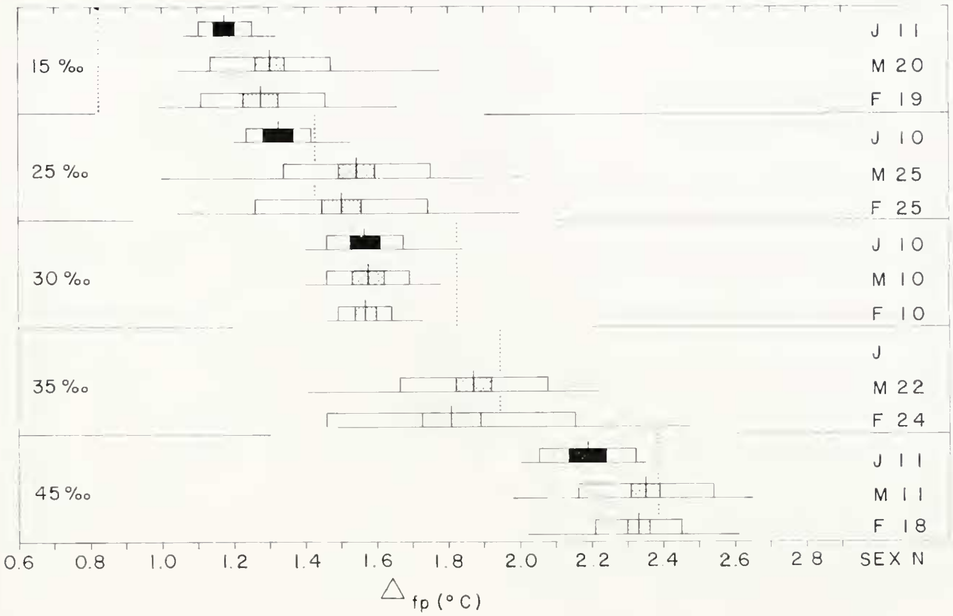

FIGURE 2. The means, standard deviations and $t_{0.05}$ standard errors for freezing point depressions of blood of juvenile (J), male (M) and female (F) sand shrimp exposed to five salinities for longer than 4 days at 15° C. Number (N) of shrimp sampled is indicated in right margin. Dotted lines indicate mean freezing point depressions of 5 salinities.

Within 8 hours after the transfer from 30‰ to 15‰, juveniles showed less resistance to the change than did adult shrimp as their blood osmoconcentration decreased immediately after transfer. Adult blood concentration increased initially to a level higher than that of the acclimation seawater and then gradually decreased. A period of oscillation followed until a new steady state was reached at ≥96 hours. The magnitude of the range, standard deviation and standard error for each group oscillated throughout the sampling period with an observed maximum variability at 8, 8 and 12 hours for juveniles, males and females, respectively.

In the transfer from 30‰ to 45‰, all shrimp showed a gradual increase in blood osmoconcentration. Juveniles exhibited an over-compensation at 24-hours prior to hyperregulation observed at ≥48 hours. Male shrimp were hyperregulating at 24 hours but their blood osmoconcentration gradually diminished to an isotonic state relative to the external medium. Females were not observed to hyperregulate until 48 hours. This condition was maintained in excess of 96 hours.

Total osmotic concentration

Shrimp at 5° C exhibit varying degrees of osmoregulation in response to a wide range of salinity (Fig. 1). At 25‰ there are no differences among the means of juvenile, male and female shrimp but blood osmoconcentration of adults is isotonic to the external medium while that of juveniles is slightly hyposmotic. Although there is regulation of a hyposmotic state in 30‰, there are again no significant differences among means of the shrimp.

There is a 1% to 5% difference between the mean blood concentration of juvenile shrimp which is hypotonic to seawater of 35‰, and that of adult shrimp which exhibit a more effective hyposmotic regulation. There is no significant difference between males and females at this salinity. At 45‰, juveniles are hypotonic to the environment while adult shrimp are in an isosmotic state. Differences of 1 and 5% exist between the mean osmoconcentration of juveniles and that of the male and female shrimp, respectively.

Osmoregulation at 5° C is most obvious at 15‰ salinity, in which the blood concentration is maintained hypertonic to the external media. Adults regulate better than juveniles (1–5% difference) and females regulate significantly higher than males (5% difference) in this salinity.

The situation at 15° C (Fig. 2) is similar to that described above for 5° C. At 25‰, blood concentrations of adults (no difference between male and female) are hypertonic to that of the seawater and significantly different (1%) from that of juvenile shrimp which remain hypotonic to the environment.

All components of the population (no differences among means) are hypotonic at 30‰. Juveniles maintain hypotonicity at 45‰ but the internal environment of adult shrimp approaches isotonicity in 35‰ and is isosmotic at 45‰. No differences between the means of adults occurs at each of these salinities. In 15‰, males and females (no difference between mans) hyperregulate to a greater extent (1% difference) than do juveniles.

Temperature effects

There is no apparent or significant difference (see Figs. 1, 2) between the osmoregulatory response of juvenile shrimp at 5° and 15° C. The blood osmoconcentration, relative to the external medium, is hypertonic at 15‰, and hypotonic at salinities ≥ 25‰, regardless of the temperature.

Male shrimp hyperregulate more effectively at the higher temperature (5% difference at 15‰; 1% at 25‰; see Figs. 1, 2). In higher salinities (30–35‰), they tend to hyporegulate better at the lower temperature (1% difference at 35‰; see Figs. 1, 2).

Females are more hyperosmotic in 25‰ salinity at the warmer temperature (1% difference; see Figs. 1, 2), and they hyporegulate more effectively in cooler, 35‰ water (5% difference; Figs. 1, 2).

Discussion

Crangon septemspinosa has been shown to exercise sufficient control over its internal osmoconcentration to the extent that it is hyposmotic in normal seawater and hyperosmotic in diluted seawater. As such, it resembles other euryhaline Caridea as well as other crustacean groups such as amphipods, isopods, panaeid shrimp and grapsoid and xanthid crabs (Robertson, 1960). In this category, the sand shrimp is distinct from those marine and brackish-water Crustacea which are isosmotic in normal seawater and hyperosmotic in diluted media, and from those forms known as osmoconformers (Kinne, 1963).

Certain variations in the regulatory response to osmotic stress, which have been attributed to differences in age, size, sex and stage of life cycle for other forms

(Flügel, 1960; Williams, 1960; Kinne, 1963, 1964), are exhibited by *C. septemspinosa*. A generalization made from studies of the response to high salinities by decapods normally living in fresh water is that almost all types eventually become isosmotic with salinities appreciably higher than the level at which blood concentration is regulated in fresh water (Born, 1968). A similar response is exhibited by adult *C. septemspinosa* to salinities greater than that of normal seawater. Hyposmotic regulation in adult *C. septemspinosa* failed at 45‰ at both temperatures. Although the response of *C. crangon* to 45‰ was not tested, there were indications that the blood concentration was approaching isotonicity at 40‰ (Flügel, 1960, 1963). The failure of adult *C. septemspinosa* to regulate at these salinities can be correlated with the lethargic behavior observed at the time of sampling and the experimental mortality (Haefner, 1969). This apparent loss of hyporegulatory capacity was not observed for the juvenile forms. The different response noted between juvenile and adult shrimp in this situation may indicate that different regulatory mechanisms, such as those outlined by Born (1968), are employed.

Flügel (1960) determined that adult *C. crangon*, at 5° and 15° C, was isotonic at 27–28‰, hypertonic from 3–26‰ and hypotonic from 30–40‰. This pattern was corroborated by Hegemann (1964). Juvenile specimens of *C. septemspinosa*, at 5° and 15° C, exhibit iostonicity at lower salinity (22–23‰) than adults (25‰) at 5° C. At 15° C the isotonic point is higher (27–28‰) for adults. In this pattern and in the fact that no significant differences in the mean osmoconcentrations between male and female shrimp (except at 15‰ at 5° C) were noted, *C. septemspinosa* is similar to *C. crangon*.

The equilibration time is similar for the two species. Flügel (1960) observed that a 5-day period of adaptation was adequate for *C. crangon* as differences ($\Delta_i - \Delta_o$) reached a maximum at 3 days and leveled off at 5–7 days. Survival (Haefner, 1969) and freezing point data (Figs. 1, 2) for *C. septemspinosa* indicate that a time period ≥ 4 days is satisfactory for this species to reach a new steady state. Other Crustacea (Williams, 1960; Thompson and Pritchard, 1969) have been shown to achieve a new steady state in less time, but lack of an adequate number of examples prohibits any meaningful comparison of species at this time.

The pattern of maintaining higher blood concentrations at lower temperatures, generally exhibited by brackish-water crustaceans (Robertson, 1960) is not adhered to by *C. septemspinosa*. There is no apparent influence of temperature on juvenile forms but adults exhibit higher blood concentrations at warmer temperatures. They are, in effect, regulating more effectively in diluted seawater and less effectively in normal seawater at warmer temperatures (15° C).

Although the osmoregulatory pattern of *C. septemspinosa* seems unusual, it can be related to its biology and migratory behavior within the estuarine zone. In Maine waters, sand shrimp spend most of the year in deeper, cooler, more saline water. Thus they are subjected to salinities of 30–34‰ at lower temperatures (5° C), the experimental combination which invokes the most effective hyposmotic regulation. They appear in the shallow estuarine zone when the water temperature reaches 5–6° C, migrate into mesohaline (3–17‰) waters, and remain there as long as prevailing water temperatures do not exceed 18° C. Under these conditions the more efficient hyperosmotic regulation is operable.

The results are similar to those obtained for *Penaeus aztecus* and *P. duorarum,* in which a loss of osmoregulatory ability occurred with lowering temperature (Williams, 1960). In the penaeids, however, the juveniles experienced the same loss as the adults.

The results do not corroborate those of Flügel (1960), who observed that osmoregulatory performance in *C. crangon*, measured as $\Delta_i - \Delta_o$, was more effective in animals adapted to 5° C than those adapted to 15° C. *Crangon septemspinosa* hyperregulates better in dilute seawater (<25‰) and hyporegulates more effectively in higher salinities at 5° C rather than at 15° C.

The difference in osmoregulatory response to temperature between the two species of *Crangon* may be related to their temperature-salinity tolerance. Haefner (1969) compared the mortality of *C. septemspinosa* with data available for *C. crangon* (Broekema, 1941) and observed that the former species is more tolerant of a given salinity range at higher temperatures than is the latter. Conversely, *C. crangon* exhibited greater survival at lower temperatures. The differences noted are probably related to the geographic ranges of the species in question. The European *C. crangon* extends farther north and not nearly as far south as *C. septemspinosa.* In their respective temperature regimes, each species would be suitably adapted to migrating into the diluted estuarine zone: *C. crangon* can do this most effectively in cooler climates while *C. septemspinosa* is better suited for accomplishing this feat in warmer waters.

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Summary

1. Freezing point determinations were made on the blood of *Crangon septemspinosa* exposed to five salinities (15, 25, 30, 35, 45‰), at two temperatures (5, 15° C).

2. Sand shrimp, transferred acutely from 30‰ to either 15‰ or 45‰, experienced changes in hemolymph osmoconcentration which were related to the osmotic strength of the receiving medium. A time period of about 4 days was required for the shrimp to reach a new steady state at 5° C.

3. *Crangon septemspinosa* was observed to regulate its internal osmoconcentration to the extent that it was hyposmotic in normal seawater (30–35‰) and hyperosmotic in diluted seawater (15–25‰). In 45‰, adults were isosmotic but juveniles remained hyposmotic to the external medium.

4. There was no apparent temperature influence on the regulatory pattern of juvenile shrimp. Adults, however, exhibited high blood concentrations at 15° C than at 5° C. They were, in effect, regulating more effectively in diluted seawater and less effectively in normal seawater at the warmer temperature.

5. Osmoregulatory performance of *C. septemspinosa* was compared with that of the European species, *C. crangon.* Differences in the response to temperature between the species were discussed in terms of their geographic ranges and related temperature regimes.

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